Vol. ■■ No. ■■ ■■ 2017

Microbial population changes in patients with medication-related osteonecrosis of the jaw treated with systemic antibiotics

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Objective. This study aimed to investigate the bacterial population in patients with medication-related osteonecrosis of the jaw (MRONJ) after treatment with doxycycline and metronidazole.

Study Design. A total of 38 patients with MRONJ (age range 55-88, mean age 73 + 8.82 standard deviation) treated with doxycycline first and with metronidazole second were enrolled in this study. Two swabs were taken at the margin of the infected MRONJ lesion after applying pressure on the marginal mucosa, and visible pus was secreted. Real-time polymerase chain reaction was used to analyze 20 periopathogenic and commensal species and the total bacterial level. Bacterial counts were compared between antibiotic treatments and with a control group of orally healthy patients who didn't have periodontal pockets of more than 3 mm (n = 29) by means of a Mann-Whitney *U* test. Comparisons between the two antibiotic treatments were performed by a paired Wilcoxon signed rank test.

Results. The total bacterial level was significantly higher in the MRONJ patients treated with systemic antibiotics compared with the control group. However, significant lower bacterial amounts were found for 12 of the 20 investigated bacteria. We couldn't establish a significant advantage of metronidazole administration after doxycycline treatment.

Conclusion. Our findings suggest that the total bacterial level in MRONJ patients is higher even when treated with systemic antibiotics. The significantly different bacterial amounts of the selected species suggest an alteration in the microbial population. (Oral Surg Oral Med Oral Pathol Oral Radiol 2017;■■:■■–■■)

Infection is believed to play an important role in the pathogenesis of medication-related osteonecrosis of the jaw (MRONJ), and recently studies have reported bacterial colonization of affected bone in MRONJ.[1-5]

Patients may be considered to have MRONJ if all the following characteristics are present: current or previous treatment with antiresorptive or antiangiogenic agents, exposed bone or bone that can be probed through an intraoral or extraoral fistula in the maxillofacial region that has persisted for longer than 8 weeks, and no history of radiation therapy to the jaws or obvious metastatic disease to the jaws.[6] MRONJ involves the maxilla and mandible with preference for the mandible.[7] MRONJ adversely affects quality of life, producing significant morbidity.

There are multiple risk factors, such as tooth extraction, diabetes, tobacco use, trauma to oral tori or caused by prosthetic appliances, poor oral hygiene, malnutrition and bone manipulation.[8-11]

Route of administration, type of medication and treatment duration are other important risk factors.[6,7]

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Received for publication Apr 14, 2016; returned for revision Oct 24, 2017; accepted for publication Nov 25, 2017.

2212-4403/$ - see front matter
https://doi.org/10.1016/j.oooo.2017.11.022

Infection naturally follows on the bone exposed to the microbial flora of the oral cavity. Almost all the MRONJ specimens are colonized with microbial biofilms.[2,4,9]

Despite the fact that they are routinely exposed to oral microorganisms that consist of more than 750 bacteria, the jaws are normally resistant to colonization. Consequently, for colonization to take place, it is needed to have a combination of patient susceptibility and the presence of potentially pathogenic microorganisms.[3,12]

The source could be the spread of odontogenic or periodontal infection. Microbial biofilms associated with teeth and periodontium that gain access to the underlying compromised bone may play a critical role in the pathogenesis of MRONJ lesions.[13-15] Bone exposure during surgery or tooth extraction works as a trigger that opens the door for bacterial invasion. This could explain the strong relationship between MRONJ and dental surgery. Furthermore, the higher sensitivity of jaws to infection, compared with other bones, strengthens this hypothesis. Jawbones come in direct contact with the external environment because of the fine layer of overlying mucosa, nonstop exposure to trauma, and presence of teeth.[16] Another fact is that MRONJ is common in cancer

Statement of Clinical Relevance

This line of investigation will be critical in the future for clinical approaches to disease intervention and for targeted antimicrobial therapeutics in patients with medication-related osteonecrosis of the jaw.

patients who are receiving chemotherapy, including immunosuppressants and corticosteroids, which give greater susceptibility to dental infection. Furthermore, medical comorbidities such as diabetes are found in MRONJ patients, and studies have reported a direct correlation with chronic periodontitis and impaired wound healing, leading to bacteria-induced bone loss.[17,18]

A biofilm is a complex community of sessile bacterial and fungal organisms attached to a surface. Biofilm organisms differ substantially from their free-floating counterparts because they are characterized by a community of cells that are attached to a substrate. They are enclosed in a matrix of extracellular polymeric substances that they have produced to connect to and to communicate with each other and with the environment. Additionally they display an altered phenotype regarding growth rate, gene transcription, and antimicrobial resistance.[3,19] A microbial biofilm applies chemical communication using signaling molecules named quorum sensing compounds as well as electrical communication via nanowires.[5,20,21] Microbial biofilms are thought to play a part in the pathogenesis of 65%-80% of all chronic infections.[4] Complex mixed-species biofilms have been reported and studied in many diseases, including dental caries, chronic periodontitis, and apical periodontitis.[14,15] Even though the presence of bacterial biofilms may not directly induce necrosis, it seems to have an important contributing role in MRONJ.

The clinical problem with MRONJ is a chronic microbial biofilm infection of bone, in the context of antiresorptive or antiangiogenic agents.[5]

Classic methods of screening and culturing for infectious disease commonly miss biofilm bacteria because these techniques are based on planktonic bacterial growth. Oral bacteria have evolved over millions of years in mixed biofilm populations, and these organisms are often unculturable. Additionally, cultures from the oral cavity have high rates of contamination, and exposure to oxygen kills a lot of the anaerobic bacteria. Biofilm identification requires direct visualization methods with advanced microscopy or DNA-and RNA-based techniques.[5]

One of the central studies that supports the concept that bone containing bisphosphonates is more sensitive to bacterial colonization was performed by Ganguli et al.[17] who reported that bacterial adhesion to pamidronate-coated hydroxyapatite (HA) was 60-fold greater than uncoated HA and 90-fold greater than clodronate-coated HA. Because clodronate is a chlorine-based bisphosphonate and not a nitrogen-based compound like pamidronate or most other bisphosphonates, these data suggest that the nitrogen moiety of the bisphosphonate compound may play a role in the pathogenesis of MRONJ. The structure of these bisphosphonate compounds could play an important role in the disease process of MRONJ.[18]

Initially it was thought that osteonecrosis was bisphosphonate related, but recently there have been reports of MRONJ with denosumab, a RANKL antibody and very strong antiresorptive.[22,23] These findings not only expand the list of drugs associated with MRONJ, they change our views on the pathophysiology of this chronic disease. Although they have different mechanism of action, denosumab and bisphosphonates both reduce the rate of bone loss, an effect that might be crucial in the etiology of MRONJ.

Several pathogenic bacteria found in the oral cavity can invade jawbones and cause bone destruction through different direct and indirect mechanisms. There are many suggested mechanisms, including direct damage to the noncellular bone matrix by bacterial acids and proteases, interaction of bacterial factors directly with bone cells, or indirectly through inflammatory agents, resulting in bone degradation and impairment of bone formation processes. Another mechanism is the invasion of osteoblasts by bacteria, producing functional disturbances and apoptosis, leading to inhibition of bone remodeling.[24] Bacterial chemical mediators of bone resorption consist of proteins such as porins and collagen-degrading enzymes, by which they obtain fundamental amino acids for growth or create an anaerobic environment in the bone for further growth and spread.[25,26]

Infection playing an important role in the pathogenesis of MRONJ could imply the need for more rational antibiotic therapy, with an efficient system of application of antibiotics to the hypovascular and hypocellular bone. That is, it should be taken into account that systemic antibiotic therapy may have a limited effect on the bacterial population associated with MRONJ lesions.[16] Here, we report the total bacterial level and the amount of selected oral bacterial phylotypes that colonize the jawbone of stage 2 and stage 3 MRONJ patients treated with systemic antibiotics and antimicrobial rinses. We also investigate the advantage of metronidazole administration after doxycycline treatment. We used real-time polymerase chain reaction (RT-PCR) analyses for 20 selected periopathogenic and commensal species.

MATERIAL AND METHODS

Sample collection

The study received approval from the Ethical Committee of the University Hospitals of the Catholic University of Leuven (S57399), was registered in the Institutional Clinical Trials database, and was conducted according to the ICH-GCP (International Conference on Harmonization Guidelines on Good Clinical Practice) principles and Helsinki guidelines. The study took place in 2015.

A total of 38 patients (male and female) with a history of intravenous bisphosphonate or denosumab therapy and bone exposure in the oral cavity for more than 8 weeks, as per the definition of MRONJ, were recruited for this

study. Only patients with stage 2 or 3 MRONJ lesions were included. Exclusion criteria were (1) any history of radiation therapy to the head and neck region and (2) obvious metastatic disease of the jaws. Written informed consent was obtained from all the 38 patients selected for this study. All the patients were treated with an oral antimicrobial rinse (chloramine 0.5% dissolved in water) in combination with oral antibiotic therapy. Two swabs were taken at the margin of the infected MRONJ lesion after applying pressure on the marginal mucosa, and visible pus was secreted. There were no periodontal conditions in this patient group. The first swab was taken after at least 2 weeks of doxycycline therapy (100 mg once a day) and the second swab was taken after 2 weeks of metronidazole therapy (500 mg three times a day). We were unable to take a second swab from seven patients who were lost to follow-up. All samples were used for RT-PCR analyses for 20 selected periopathogenic and commensal species (n = 38 for the doxycycline group; n = 31 for the metronidazole group). As control group, the database of Loozen et al.[27] with the microbiologic analyses of untreated healthy patients was used. Subgingival plaque samples were taken by dental clinicians with access to case history, radiographs, and clinical data. Clinicians had a choice to take between 1 and 5 samples using sterile paper points. These paper points were deposited into marked Eppendorf tubes and sent at room temperature for RT-PCR analyses for the 20 selected pathogenic and commensal species. The control group consisted of patients who didn't have pockets of more than 3 mm; these patients weren't treated with antibiotics and didn't have MRONJ (n = 29 for control group). The oral flora in these shallow pockets was considered to be equal to the normal oral flora in healthy patients.

DNA extraction and RT-PCR

After taking the swabs, samples were transported at room temperature to Advanced Dental Diagnostics B.V. (Malden, the Netherlands). DNA from the samples was obtained using the Quickextract DNA extraction (Epicentre Biotechnologies, Madison, WI, USA) according to the manufacturer's instructions. We used RT-PCR analyses for 20 selected oral bacteria. The bacterial profile was determined by RT-PCR by 16 S rRNA gene fragments. The targets were detected by using TaqMan probes (Table I). Total and individual levels of selected periopathogenic and commensal species (n = 20) were calculated. This analysis provides information of both the quantity (total bacterial level) and the quality (composition) of the biofilm. The original microbial data were converted to log10 values. The total amount of bacteria and the relative presence of the different bacteria were calculated.

Table I. List of oral bacteria

Abbreviation	*Genus and species*
Aa	*Aggregatibacter actinomycetemcomitans*
Pg	*Porphyromonas gingivalis*
Tf	*Tannerella forsythia**
Td	*Treponema denticola*
Pi	*Prevotella intermedia*
Fn	*Fusobacterium nucleatum*
Pm	*Parvimonas micra*†
Pn	*Prevotella nigrescens*
Cg	*Campylobacter gracilis*
Cr	*Campylobacter rectus*
En	*Eubacterium nodatum*
Ec	*Eikenella corrodens*
Cs	*Capnocytophaga* species
Cc	*Campylobacter concisus*
Smg	*Streptococcus mitis* group
Sg	*Streptococcus gordonii*
Sc	*Streptococcus constellatus*
Ao	*Actinomyces odontolyticus*
Av	*Actinomyces viscosus*
Vp	*Veillonella parvula*

*Previously *Tannerella forsythensis.*
†Previously *Peptostreptococcus micros.*

Statistical analyses

Bacterial counts were log10 transformed before analysis. To be able to deal with zero counts, the log10 transformation was taken from the bacterial count + 1.

Bacterial counts were compared between antibiotic treatments and with a control group of patients who didn't have periodontal pockets of more than 3 mm by means of a Mann-Whitney *U* test. Comparisons between the two antibiotic treatments were performed by a paired Wilcoxon signed rank test.

Antibiotic treatments were also compared with each other with respect to absence or presence of the various bacterial species by means of a generalized linear mixed model for binary data with patient as random factor. Comparisons with the group of patients who didn't have pockets of more than 3 mm were performed by means of a generalized linear model for binary data. Each time, a logit link was applied. Comparisons between antibiotic treatments and the group of shallow pockets were corrected for simultaneous hypothesis testing according to Sidak. Statistical significance was accepted at P values < .05.

RESULTS

The study included 20 men and 18 women. The age range was 55-88, the mean age was 73 + 8.82 standard deviation (SD). Six patients were treated for osteoporosis and 32 patients were treated for cancer; 20 patients were treated with intravenous (IV) bisphosphonates, 10 patients received denosumab, and 8 patients had a history

Table II. Demographic and clinicopathologic features of study patients

Number	Age (2015)	Gender	Illness Requiring ONJ-Inducing Medication	ONJ-Inducing Medication
1	80	F	Osteoporosis	BP
2	76	F	Multiple myeloma	BP
3	75	M	Osteoporosis	BP
4	60	M	Renal cancer	BP + Denosumab
5	83	M	Multiple myeloma	BP
6	68	F	Breast cancer	Denosumab
7	75	F	Multiple myeloma	BP
8	64	F	Multiple myeloma	BP
9	74	F	Breast cancer	Denosumab
10	79	M	Prostate cancer	BP
11	65	F	Breast cancer	Denosumab
12	88	F	Breast cancer	BP
13	81	F	Osteoporosis	BP + Denosumab
14	80	F	Renal cancer	BP + Denosumab
15	71	F	Osteoporosis	Denosumab
16	79	F	Breast cancer	BP
17	66	M	Multiple myeloma	BP
18	67	M	CML	BP
19	77	M	Multiple myeloma	BP
20	65	F	Multiple myeloma	BP
21	88	M	Prostate cancer	Denosumab
22	59	F	Breast cancer	BP + Denosumab
23	64	F	Osteoporosis	BP
24	70	M	Prostate cancer	BP + Denosumab
25	55	M	Stomach cancer	Denosumab
26	69	M	Prostate cancer	Denosumab
27	60	M	Renal cancer	Denosumab
28	82	M	Multiple myeloma	BP
29	82	M	Renal cancer and prostate cancer	BP + Denosumab
30	81	M	Prostate cancer	Denosumab
31	84	M	Prostate cancer	BP + Denosumab
32	67	F	Breast cancer	BP
33	73	M	Multiple myeloma	BP
34	64	F	Osteoporosis	BP
35	84	F	Multiple myeloma	BP
36	80	M	Prostate cancer	BP
37	62	M	Lung cancer	BP
38	77	M	Prostate cancer	Denosumab

BP, bisphosphonates; *CML,* chronic myeloid leukemia; *ONJ,* osteonecrosis of the jaw.

of treatment with the combination of IV bisphosphonates and denosumab (Table II).

Total bacterial level

There were was a significant difference in the total bacterial count of the doxycycline (mean 8.256 ± 0.932 SD) and metronidazole (mean 8.29 ± 0.909 SD) samples in the MRONJ patients compared with the control group of healthy patients who didn't have pockets of more than 3 mm (mean 6.659 ± 1.364 SD). The total bacterial level was significantly higher in the MRONJ patients treated with these antibiotics (Figure 1).

Doxycycline treatment

Significant lower bacterial amounts were found for *Aggregatibacter actinomycetemcomitans* (Aa), *Treponema denticola* (Td), *Prevotella intermedia* (Pi), *Fusobacterium nucleatum* (Fn), *Capnocytophaga* species (Cs), *Streptococcus mitis* group (Smg), *Streptococcus gordonii* (Sg), *Actinomyces odontolyticus* (Ao), *Actinomyces viscosus* (Av), and *Veillonella parvula* (Vp) in the doxycycline group compared with the control group ($P < .0002$, $P < .0001$, $P < .002$, $P < .0001$, $P < .0001$, $P < .002$, $P < .0001$, $P < .0001$, $P < .0001$, and $P < .0002$, respectively).

Metronidazole treatment

Significant differences were found for Aa, *Tannerella forsythia* (Tf), Td, Pi, Fn, *Campylobacter gracilis* (Cg), Cs, Smg, Sg, *Streptococcus constellatus* (Sc), Av, and Vp between the metronidazole group and the control group ($P < .0004$, $P < .02$, $P < .0001$, $P < .001$, $P < .0001$, $P < .0001$, $P < .0001$, $P < .003$, $P < .004$, $P < .0001$ and $P < .0001$, respectively). Significantly lower amounts were found for all these bacteria except for Sc, where a significantly higher amount was found in the metronidazole group.

Differences between doxycycline and metronidazole treatment

Comparisons between the 2 antibiotic treatments were performed by a paired Wilcoxon signed rank test. Significant differences between the doxycycline and metronidazole groups were found for Fn, Cg, and Ao. Significantly lower amounts of Fn and Cg were found in the group treated with metronidazole compared with the doxycycline group ($P < .04$ and $P < .005$, respectively). However, significantly higher amounts of Ao were found in the metronidazole group compared with the patients treated with doxycycline ($P < .04$). No significant differences were noted between doxycycline and metronidazole treatment for the other selected bacteria (Figure 2).

Comparison of absence or presence of bacterial species

Antibiotic treatments were also compared with each other and with the control group with respect to absence or presence of the various bacterial species by means of a generalized linear mixed model for binary data with patient as random factor. The presence in the samples was significantly less for the group treated with doxycycline compared with the control group for Aa, Td, Pi, Cs, Smg, Sg, and Av ($P < .02$, $P < .0002$, $P < .01$, $P < .0004$, $P < .003$, $P < .01$, and $P < .0002$, respectively). Furthermore, a significant lower incidence of Aa, Td, Pi, Cs, Smg, Sg, Av, and Vp was also found in the group

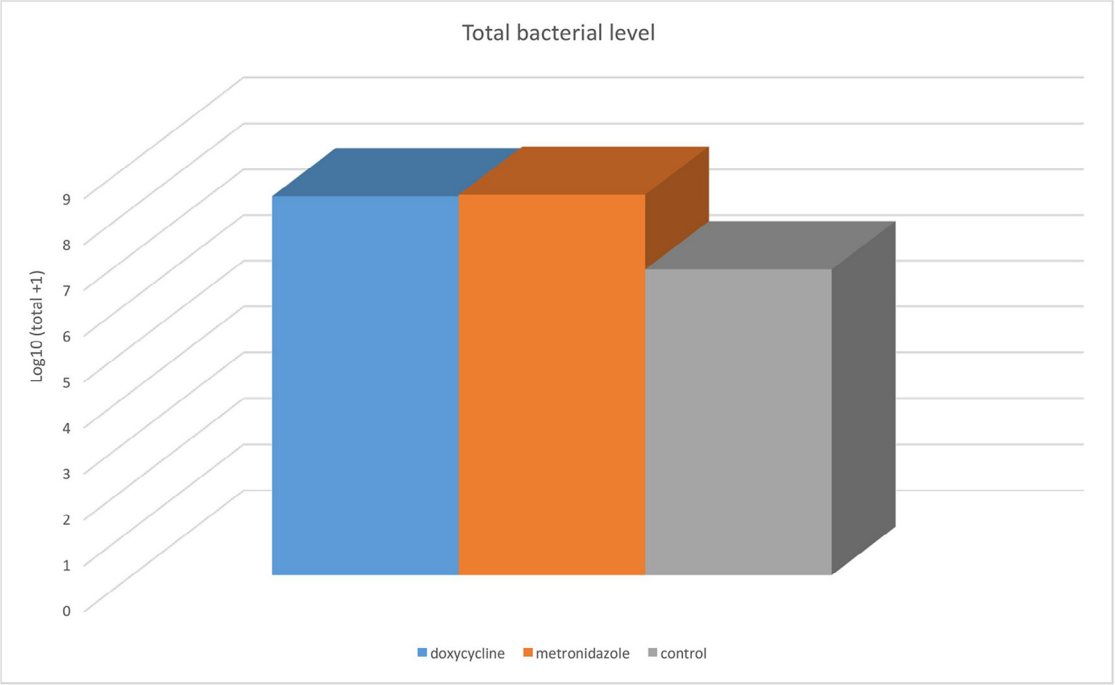

Fig. 1. Total bacterial level in the medication-related osteonecrosis of the jaw patients treated with doxycycline and metronidazole and in the control group.

treated with metronidazole compared with the control group ($P < .03$, $P < .0002$, $P < .01$, $P < .0006$, $P < .01$, $P < .01$ $P < .0004$, and $P < .05$, respectively).

Antibiotic treatments were also compared with each other with respect to absence or presence of the various bacterial species (Figure 3). There was a significant lower incidence of Tf, Fn, *Eubacterium nodatum* (En), Sc, and Av in the metronidazole group compared with the group treated with doxycycline ($P < .02$, $P < .0001$, $P < .0001$, $P < .02$, and $P < .002$, respectively).

DISCUSSION

The paucity of investigation into the role of oral bacteria in the pathogenesis of MRONJ has hindered proper disease characterization and treatment. There are many hypotheses on MRONJ pathogenesis such as remodeling suppression that allows accumulation of nonviable osteocytes, direct cytotoxic effect on osteocytes, antiangiogenic effects, and the role of oral bacteria.[8] Factors such as dental infections, trauma, and invasive procedures act as initiators of MRONJ.[28] It is known that several species of bacteria can cause alveolar bone destruction by their products (e.g., lipopolysaccharides).[25] It is not known whether bacteria promote the lesion, or whether they colonize the MRONJ lesion after it has developed. This study using 16 S rRNA culture-independent molecular methods determined the bacterial profile of MRONJ patients treated with systemic antibiotics. We found a significantly higher total bacterial count in the MRONJ patients treated with doxycycline and metronidazole compared with a control group of healthy untreated patients.

Aggregatibacter actinomycetemcomitans is considered as an opportunistic pathogen and has been reported to be associated with periodontal disease and osteomyelitis, as well as MRONJ.[1,2,29] Most of these assumptions are based only on microscopic observations. We used molecular 16 S rRNA gene techniques, with RT-PCR analyses, but did not observe *Aggregatibacter actinomycetemcoitans*.

When comparing our results to the color-coded biofilm complexes described by Socransky et al.,[30] we found that the red complex pathogenic anaerobic bacteria (Tf, Td, and *Porphyromonas gingivalis* [Pg]) didn't have a clear association with disease in our study.[27] On the contrary, significantly lower bacterial amounts of Td were found in patients treated with doxycycline and significantly lower amounts of Tf and Td were found in the metronidazole treatment group. Additionally, our study also found a lower prevalence of Pi, Fn, and Cg. These bacteria are members of the orange complex bacteria, which was reported to be strongly correlated with the red complex and with disease in the study of Socransky et al.[30] Different bacteria displayed a decreased abundance in our study:

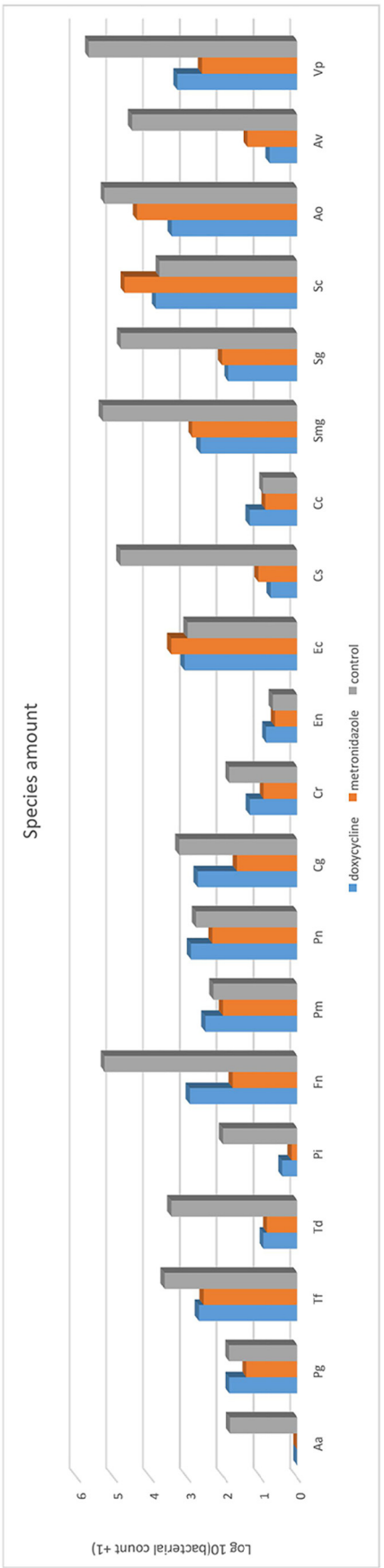

Fig. 2. Polymerase chain reaction–quantified amounts of the 20 selected bacteria in the medication-related osteonecrosis of the jaw patients treated with doxycycline and metronidazole and in the control group.

Cs, Smg, Sg, Sc, Av, Ao, and Vp. These species groups have been defined as non–disease associated, situated in the green, yellow, or purple complexes.

This finding suggests an alteration in the microbial population. Although all the MRONJ patients in our study had clinical signs of infection, we did not find high numbers of periodontal pathogens such as Pg, Tf, and Td.

Cancer patients are more susceptible to infection because they are immunocompromised and have reduced regenerative capability, whereas osteoporotic patients have disturbed levels of bone regeneration.[31] Osteoporotic patients often suffer from other chronic diseases and have increased risk of developing opportunistic infections, which may relate to reduced bone mineral density and alveolar bone loss, premature teeth loss, and increased severity of periodontal diseases.[1,32]

Metronidazole is one of the most commonly indicated antibiotics for the treatment of periodontal disease.[2] However, we couldn't find an advantage of metronidazole administration after doxycycline treatment in patients with stage 2 MRONJ. It is well established that the long-term use of antimicrobials might eradicate normal flora, which induces an imbalance in microbial composition.[5] A limitation of the study is that only 20 oral bacteria are examined. Because the oral cavity can harbor more than 1000 microorganisms, other bacteria can also play a crucial role. However, the 20 bacteria investigated in the present study are all key bacteria in the oral environment and have proven to play a crucial role in the oral cavity.[30] Additionally, metagenomic identification of bacteria determines the presence of specific DNA but not viability; because bacterial DNA can remain PCR detectable for up to 1 year after cell death, metagenomics may overestimate current bacterial load.[33] Further, a single microbiologic examination cannot determine if specific bacteria are permanent or transient residents or are primary pathogens or merely bystanders to disease.

Our study using 16 S rRNA molecular technique indicates an alteration in the microbial population of MRONJ patients treated with systemic antibiotics. However, we did not compare these results with untreated MRONJ patients because of the ethical problems with not treating patients. No significant advantage was identified for metronidazole administration after doxycycline treatment. These findings require further evaluation with larger participant populations and more oral microorganisms have to be examined. Continuing in this line of investigation will be critical in the future for clinical approaches to disease intervention and for targeted antimicrobial therapeutics.

The authors are grateful to professor W. Teughels and Advanced Dental Diagnostics B.V. for providing the microbiologic data set on which analysis was performed.

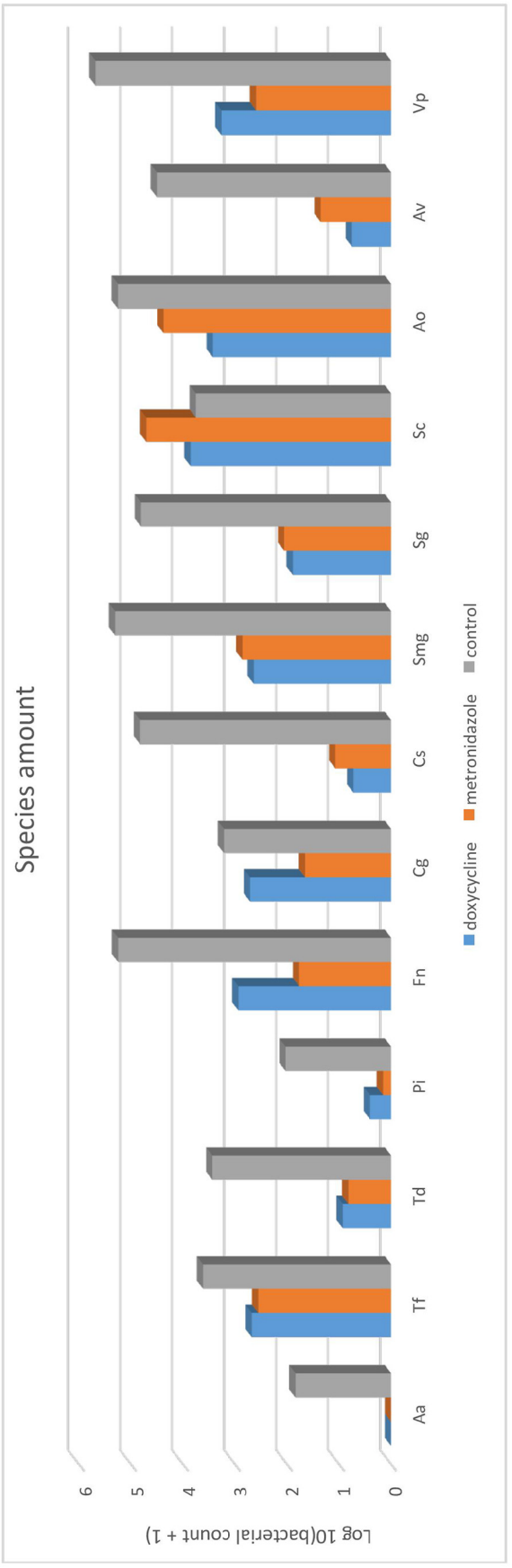

Fig. 3. Bacterial species with significant different counts in medication-related osteonecrosis of the jaw patients treated with doxycycline and metronidazole compared with the control group.

REFERENCES

1. Hansen T, Kunkel M, Weber A, Kirkpatrick J. Osteonecrosis of the jaws in patients treated with bisphosphonates—histomorphologic analysis in comparison with infected osteoradionecrosis. *J Oral Pathol Med*. 2006;35:155-160.
2. Hansen T, Kunkel M, Springer E, et al. Actinomycosis of the jaws—histopathological study of 45 patients shows significant involvement in bisphosphonate-associated osteonecrosis and infected osteoradionecrosis. *Virchows Arch*. 2007;451:1009-1017.
3. Sedghizadeh PP, Kumar SK, Gorur A, Schaudinn C, Shuler CF, Costerton JW. Microbial biofilms in osteomyelitis of the jaw and osteonecrosis of the jaw secondary to bisphosphonate therapy. *J Am Dent Assoc*. 2009;140:1259-1265.
4. Sedghizadeh P, Kumar S, Gorur A, Schaudinn C, Shuler C, Costerton W. Identification of microbial biofilms in osteonecrosis of the jaws secondary to bisphosphonate therapy. *J Oral Maxillofac Surg*. 2008;66:767-775.
5. Kumar S, Gorur A, Schaudinn C, Shuler C, Costerton W, Sedghizadeh P. The role of microbial biofilms in osteonecrosis of the jaw associated with bisphosphonate therapy. *Curr Osteoporos Rep*. 2010;8:40-48.
6. Ruggiero S, Dodson T, Fantasia J, et al. American Association of Oral and Maxillofacial Surgeons Position Paper on Medication-Related Osteonecrosis of the Jaw—2014 update. *J Oral Maxil Surg*. 2014;72:1938-1956.
7. Jacobsen C, Metzler P, Obwegeser JA, Zemann W, Graetz KW. Osteopathology of the jaw associated with bone resorption inhibitors: what have we learned in the last 8 years? *Swiss Med Wkly*. 2012;142:w13605.
8. Allen MR, Burr DB. The pathogenesis of bisphosphonate-related osteonecrosis of the jaw: so many hypotheses, so few data. *J Oral Maxillofac Surg*. 2009;67:61-70.
9. Marx R, Sawatari Y, Fortin M, Broumand V. Bisphosphonate-induced exposed bone (osteonecrosis/osteopetrosis) of the jaws: risk factors, recognition, prevention, and treatment. *J Oral Maxillofac Surg*. 2005;63:1567-1575.
10. Ji X, Pushalkar S, Li Y, Glickman R, Fleisher K, Saxena D. Antibiotic effects on bacterial profile in osteonecrosis of the jaw. *Oral Dis*. 2012;18:85-95.
11. Thumbigere-Math V, Sabino MC, Gopalakrishnan R, et al. Bisphosphonate-related osteonecrosis of the jaw: clinical features, risk factors, management, and treatment outcomes of 26 patients. *J Oral Maxillofac Surg*. 2009;67:1904-1913.
12. Boff R, Salum F, Figueiredo M, Cherubini K. Important aspects regarding the role of microorganisms in bisphosphonate-related osteonecrosis of the jaws. *Arch Oral Biol*. 2014;59:790-799.
13. Dunstan C, Felsenberg D, Seibel M. Therapy insight: the risks and benefits of bisphosphonates for the treatment of tumor-induced bone disease. *Nat Clin Prac Oncol*. 2007;4:42-55.
14. Siqueira JF, Rôças IN. Community as the unit of pathogenicity: an emerging concept as to the microbial pathogenesis of apical periodontitis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2009;107:870-878.
15. Kuramitsu HK, He X, Lux R, Anderson MH, Shi W. Interspecies interactions within oral microbial communities. *Microbiol Mol Biol Rev*. 2007;71:653-670.
16. Kos M, Luczak K. Bisphosphonates promote jaw osteonecrosis through facilitating bacterial colonisation. *Biosci Hypotheses*. 2009;2:34-36.
17. Ganguli A, Steward C, Butler SL, et al. Bacterial adhesion to bisphosphonate coated hydroxyapatite. *J Mater Sci Mater Med*. 2005;16:283-287.
18. Diel I, Fogelman I, Al-Nawas B, et al. Pathophysiology, risk factors and management of bisphosphonate-associated osteonecrosis of the jaw: is there a diverse relationship of amino- and non-aminobisphosphonates? *Crit Rev Oncol Hematol*. 2007;64:198207.
19. Donlan R, Costerton W. Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin Microbiol Rev*. 2002;15:167-193.
20. Hall-Stoodley L, Stoodley P. Evolving concepts in biofilm infections. *Cell Microbiol*. 2009;11:1034-1043.
21. Wanger G, Gorby Y, El-Naggar M, et al. Electrically conductive bacterial nanowires in bisphosphonate-related osteonecrosis of the jaw biofilms. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2013;115:71-78.
22. Pazianas M, Miller P, Blumentals W, Bernal M, Kothawala P. A review of the literature on osteonecrosis of the jaw in patients with osteoporosis treated with oral bisphosphonates: prevalence, risk factors, and clinical characteristics. *Clin Ther*. 2007;29:1548-1558.
23. Pazianas M. Osteonecrosis of the jaw and the role of macrophages. *J Natl Cancer Inst*. 2011;103:232-240.
24. Henderson B, Nair S. Hard labour: bacterial infection of the skeleton. *Trends Microbiol*. 2003;11:570-577.
25. Nair S, Meghji S, Wilson M, Reddi K, White P, Henderson B. Bacterially induced bone destruction: mechanisms and misconceptions. *Infect Immun*. 1996;64:2371-2380.
26. Harrington D. Bacterial collagenases and collagen-degrading enzymes and their potential role in human disease. *Infect Immun*. 1996;64:1885-1891.
27. Loozen G, Ozcelik O, Boon N, et al. Inter-bacterial correlations in subgingival biofilms: a large-scale survey. *J Clin Periodontol*. 2014;41:1-10.
28. Otto S, Schreyer C, Hafner S, et al. Bisphosphonate-related osteonecrosis of the jaws—Characteristics, risk factors, clinical features, localization and impact on oncological treatment. *J Craniomaxillofac Surg*. 2012;40:303-309.
29. Naik N, Russo T. Bisphosphonate-related osteonecrosis of the jaw: the role of actinomyces. *Clin Infect Dis*. 2009;49:1729-1732.
30. Socransky SS, Haffajee AD, Cugini MA, Smith K. Microbial complexes in subgingival plaque. *J Clin Periodontol*. 1998;25:134-144.
31. Ruggiero S, Gralow J, Marx R, et al. Practical guidelines for the prevention, diagnosis, and treatment of osteonecrosis of the jaw in patients with cancer. *J Oncol Pract*. 2006;2:7-14.
32. Vahtsevanos K, Kyrgidis A, Verrou E, et al. Longitudinal cohort study of risk factors in cancer patients of bisphosphonate-related osteonecrosis of the jaw. *J Clin Oncol*. 2009;27:5356-5362.
33. Sedghizadeh P, Yooseph S, Fadrosh D, et al. Metagenomic investigation of microbes and viruses in patients with jaw osteonecrosis associated with bisphosphonate therapy. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2012;114:764-770.

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